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Changes in the quantitative and qualitative characteristics of seedless barberry (*Berberis Vulgaris* L.) fruit as influenced by fruit thinning

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ABSTRACT

Purpose: Barberry has been used for many centuries due to its highly nutritious benefits, ornamental value, and medicinal properties. Iran is the largest producer of seedless barberry and this has been growing in various regions with dry climates, poor soil conditions and severe water shortages. Alternative bearing is a frequent problem in seedless barberry production. To avoid it, thinning has been employed as a common cultural practice in orchard management. **Research method:** In this study, three chemical thinners including gibberellic acid (GA_3) at 75, 100 and 150 mg/L, naphthalene acetic acid (NAA) at 10, 20 and 40 mg/L and ethephon at 50, 100 and 200 mg/L, and hand thinning (20%) were applied in a commercial orchard in Birjand, Iran, one week after petal fall. Thinning rate, quantitative and qualitative traits were investigated in comparison to the control in the split plot arrangement in a randomized complete block design during 2015 and 2016. **Findings:** Results showed that NAA at 10 mg/L caused the highest fruit abscission. Vegetative traits such as shoot length, number of leaves per shoot and leaf area showed significant increases under the treatments while shoot diameter had no significant difference with control. Chemical thinning remarkably enhanced the starch and sugar of the shoots, especially in "on" year (2015). The minimum chlorophyll content in "off" year was observed in control and the highest by NAA at 10 ppm. All the treatments increased seedless barberry shrub yield in the "off" year (2016). Biochemical traits such as ascorbic acid, total soluble solids, titratable acidity and anthocyanin improved in most treatments. **Research limitations**: No limitations were encountered**. Originality/Value:** To avoid alternative bearing in seedless barberry shrubs, thinning has been employed as a common cultural practice in orchard management. So, the application of NAA 10 mg/l is recommended for control of alternative bearing and also better fruit quality.

INTRODUCTION

An important aspect of horticulture is the cultivation of plants for food, fiber, biofuel, medicine, and other products used to sustain and enhance human life (Vijayan et al., 2008; Sengul et al., 2011). Seedless barberry (*Berberis vulgaris* L.) is an important underutilized horticultural crop that belongs to the family of Berberidaceae and is native to Iran (Zargari, 1990). Iran is the largest producer of seedless barberry with a production quantity of more than 22322 tons of dried berries in around 19220 ha (Ahmadi et al., 2021). Most of the consumption of seedless barberry is in a form of dried berries. Seedless barberry is usually propagated by two- or three-year-old suckers or stem cutting and It can be grown in any soil especially in poor and alkaline ones with pH up to 8.5 and very drought and salinity tolerant in which most of the other trees cannot produce satisfactory fruits (Alemardan et al. 2013). Apart from its agronomical value, it has been used in landscape design, plant disease control, textile dying (Alemardan et al., 2013) and for its medicinal properties such as anticancer, antimicrobial (Alimirzaee et al., 2009), decreasing addiction and dependency to morphine (Hashemzaei et al., 2012) and rehabilitation, hypoglycemic, anti-diabetic (Shidfar et al., 2012) and reducing LDL and total cholesterol (Ebrahimi-Mamaghani et al., 2009).

One major problem in seedless barberry production is alternate bearing (Kafi et al., 2002). In fruit trees, the alternate bearing mechanism shows that in years with a higher yield, fewer branches with generative buds are produced. Lack of sufficient nutrients to produce flower buds or phytohormones imbalance are the major causes of alternate bearing, Intense frequency in productivity in this crop results in a delay in fruit maturity and the loss of fruit size and crop quality. To control alternate bearing in fruit trees, a technique called thinning is used. In fruit trees, if an extra fruit set happens, the insufficient resources cannot support the demands of fruit lets leading to the production of low-quality fruits and alternate bearing. To avoid these phenomena fruit thinning is an essential orchard cultural practice to improve the partitioning of photosynthetic resources and tree performance (Costa et al., 2019). In this method, excessive flowers or young fruit lets are eliminated from branches (Taghipure et al., 2011). Fruit thinning is usually employed by hand thinning which is accurate but laborious, expensive and slow or by chemical thinning which is easier and more promising but not environmentally friendly (Costa et al., 2019).

In an experiment on the effects of GA_3 and ethephon on seedless barberry, it was revealed that these chemicals can reduce the alternate bearing (Balandary, 1995). Milić et al. (2018) reported that both naphthalene acetic acid (NAA) and benzyl adenine (BA) stimulated vegetative growth and the development of leaf area in northern high-shrub blueberries. NAA treatment at 20 and 40 mg/L two weeks after anthesis significantly increased the thinning of apricot 'Khiary' fruit (Taghipure & Rahemi, 2009).

There is little information regarding the alternate bearing of seedless barberry and the feasibility of using hand thinning technique and chemicals thinners application for berry thinning. Therefore, the present study aimed to study the effects of hand thinning, NAA, GA_3 and ethephon for fruit thinning and to investigate their influences on vegetative growth, fruit quality and yield of seedless barberry fruit.

MATERIALS AND METHODS

Experimental site

This study was carried out in an orchard on 12 km of Birjand-Kerman road (latitude 32°56' N., longitude 59°13' E., elevation 1480 m.) with arid and semi-arid climate under the natural conditions in 2015 ("on" year) and 2016 ("off" year), Birjand, Iran. Irrigation in this orchard was carried out over, every 14 days with a surface irrigation system. The result of the soil analysis is shown in Table 1. Mineral deficiencies are compensated with fertilization during the growing season.

Plant material and treatments

A similar 15-year-old shrub in terms of vegetative and reproductive characteristics was used in this experiment. The following treatments were applied on all branches was performed: hand thinning (20% of fruit lets removed by hand), NAA (10, 20 or 40 mg/L, Merck, Germany), GA_3 (75, 100 or 150 mg/L, Merk, Germany), ethephon (50, 100 or 200 mg/L, Merck, Germany) and untreated control. For each treatment, five shrubs were used and the density in the orchard was 2 × 3m. One week after the petal fall in early spring, the shrubs were sprayed with a backpack sprayer in the afternoon, with the amount of solution of 2.8 L per each treatment. From each shrub, four branches which had similar lengths and diameters were selected and labelled to measure quantitative and qualitative traits. The same shrubs were subjected to the same treatments in both experimental years.

Fruit drop percentage and vegetative growth characteristics

Berries drop percentage (overall fruit set) was obtained by calculating the berries number at four clusters before treatment and the final stage of ripening. At the end of the growing season, four one-year-old branches per shrub which were similar in all shrubs were selected and the length, diameter and number of leaves in each shoot were measured. To measure the leaf area (LA), 20 leaves were taken from the middle part of the shoots from each shrub (80 leaves in total per treatment). The LA was measured using Image J (v.1.51f) software (Easlon & Bloom, 2014).

Shoot sugar and starch and chlorophyll content

Shoot sugar and starch were calculated at the end of the growing season. The concentration of starch was estimated with the procedure described by Hedge and Hofreiter (1962) and the light absorption of the solution was read using a spectrophotometer (UV-Win X- ma 2000) at 630 nm. Dissolved sugar was calculated using the phenol and sulfuric acid method introduced by Kochert (1978) at 485 nm. The content of leaf chlorophyll was estimated with the procedure described by Lichtenthder (1987) using a spectrophotometer (UV-Win X- ma 2000) at 646.6 and 663.6 nm.

Yield per shrub

To calculate the yield of each branch, all berries of each branch were harvested separately. To measure the fresh weight of each shrub, the fruits of each shrub were harvested in November and weighed with a digital 0.001 g scale (model KB120-3N, Kern, the UK).

Table 1. Chemical analysis of the soil of the seedless barberry orchard in Birjand.

Texture soil	pH	Saturation (%)	EC (ds/.m)	Depth (cm)	K (ppm)	P (ppm)	N (%)
Sandy- clay	7.9	25	7.8	0-30	198	15	0.1

Fruit characteristics

To measure fruit fresh and dry weight, each sample consisted of 20 fruits randomly taken out from each shrub, making the sample 100 fruits in total per each treatment. Samples with 100 already harvested fruits were weighed with a digital 0.001g scale (model KB120-3N). Then, the fruits were oven-dried at 80°C for 24 hours to estimate their dry weight. The water displacement measurement method was used to determine the volume of 10 berries. Fruit anthocyanin was calculated using the method introduced by Wrolstad (1976) using a spectrophotometer (UV-Win X- ma 2000) at 510 and 700 nm. Total acidity (%) was determined by titration using the sodium hydroxide method (Ranjana, 1986). Ascorbic acid was measured by titration using2,6-dichlorophenol-indophenol (Kassem et al., 2011). Total soluble solids (TSS) were measured using a handheld refractometer (Model Abbe, Waj, China). Fruit acidity was measured using a digital pH meter (Model 110, Jenway, and the UK).

Statistical analysis

The experiment was a split-plot arrangement where thinning was the main plot and year was a subplot laid out in a randomized complete block design with five replicates. We used the GLM procedure of SAS statistical software for data analysis and Duncan's multiple range tests at 5% level for means comparison.

RESULTS

According to ANOVA results, the interaction effects of thinning and year were significant for all the traits except fruit drop, shoot length and diameter**,** TSS and fruit width while the simple effects of thinning or year were significant in these traits (Table 2 and 3). There wasn't a significant difference in shoot diameter in this experiment.

Fruit drop percentage

The results showed that the interaction effects of thinning and year were not significant for the fruit drop parameters while the simple effect of thinning shows the significant difference with the control. All of the chemical treatments resulted in a significant increase in fruit drop as compared to the control. The highest rate of drop was observed in 10 NAA ppm treatment that exhibited 29.9% fruit the drop and the lowest of it related to control (3.41%) (Fig. 1).

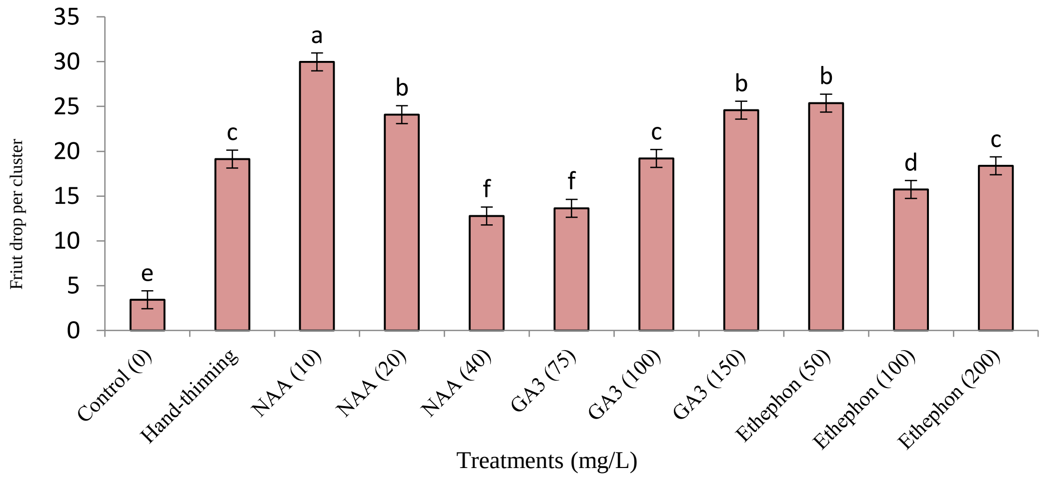

Fig. 1. Effect of different thinning techniques on fruit drops of seedless barberry. Columns with common letters are not significantly different according to Duncan's test.

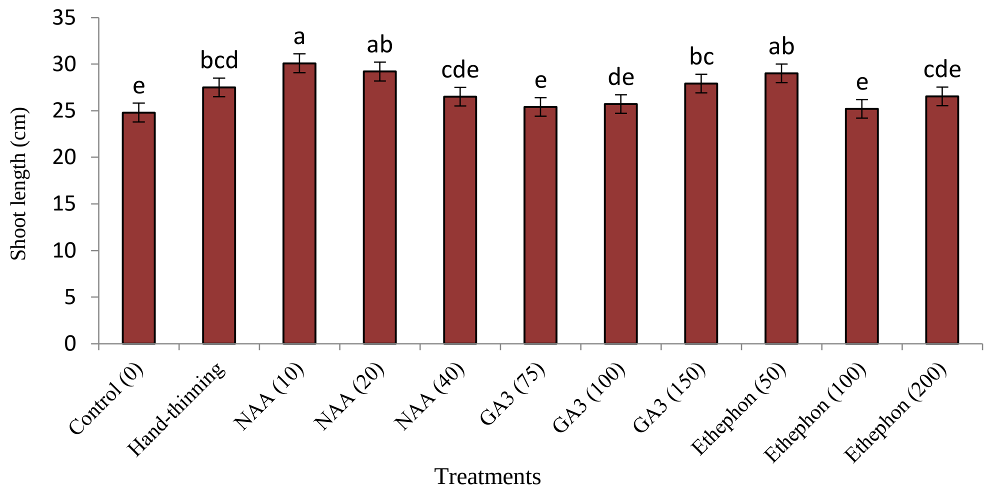

Fig. 2. Effect of different thinning techniques on shoot length in seedless barberry. Columns with common letters are not significantly different according to Duncan's test.

Table 2. Analysis of variance (ANOVA) of the effect of thinning and time on some growth and morphological parameters of barberry.

SOV	df	Shoot length	Shoot diameter	No. of leaves	Leaf area
Replication	4	1.7 ^{ns}	3.66 ^{ns}	0.22 ^{ns}	0.002^{ns}
Thinning (a)	10	712.2**	34.3 ^{ns}	251.7**	17.98**
Whole Plot Error	1	0.361	5.98	0.153	0.15
Time (year) (b)	10	32.5**	2.69 ^{ns}	29.45**	4.15**
a × b	4	1.1 ^{ns}	3.16 ^{ns}	1.61*	0.10**
r × b	40	2.5 ^{ns}	3.91 ^{ns}	0.94*	0.005^{ns}
Subplot error	4	2.25	4.13	0.364	0.31
CV		6.1	20	2.4	1.19

Table 2. (Continued).

SOV	df	Leave chlorophyll	Yield per Shoot	Fruit drop	Shoot Starch	Shoot sugar
Replication	4	0.37**	78081 ^{ns}	0.13 ^{ns}	0.88*	0.08 ^{ns}
Thinning (a)	10	25.30**	18777051**	539.04**	18.41**	5.55**
Whole Plot Error	1	0.21	359083	1.05	0.57	0.28
Time (year) (b)	10	9.89**	3280247952**	216.03**	26.86**	19.90**
a × b	4	**9.15	8395813**	2.4 ^{ns}	2.55**	**12.72
r × b	40	0.04 ^{ns}	167764 ^{ns}	3.38 ^{ns}	0.41 ^{ns}	0.11*
Subplot error	4	0.01	122981	1.91	0.28	0.04
CV		1.28	86.2	7.37	7.71	1.78

Table 3. Analysis of variance (ANOVA) of the effect of thinning and time on some growth and chemical parameters of barberry fruit.

SOV	df	Length	Width	Volume	Fresh W.	Dry W.
Replication	4	0.04**	0.24 ns	0.08ns	0.05 ns	0.35*
Thinning (a)	10	0.07**	0.94ns	0.45**	4.22**	0.95**
Whole Plot Error	1	0.10	0.21	0.28	0.99	0.88
Time (year) (b)	10	0.44**	0.45ns	1.33**	9.73**	9.16**
a × b	4	0.21**	0.61ns	0.69**	2**	0.54**
r × b	40	0.006*	0.52 ns	0.00005ns	0.06ns	0.06ns
Subplot error	4	0.002	0.27	0.12	0.03	0.09
CV		0.64	11.02	7.51	1.17	6.72

*, ** represents significant at probability levels of 0.05 and 0.01 respectively; ns means non-significant.

Table 3. (Continued).

SOV	df	Ascorbic acid	pH	TSS	TA	Anthocyanin
Replication	4	1312ns	0.08**	4.40**	0.0006ns	0.0002 ns
Thinning (a)	10	611184**	0.02**	25.02**	1.30**	0.02**
Whole Plot Error	1	1401.1	0.02	10.47	0.02	0.0004
Time (year) (b)	10	341051**	0.007*	6.44**	19.40**	0.04**
a × b	4	8100**	0.002*	0.93 ns	0.22**	**0.001
r × b	40	1086.4 ns	0.0002 ns	0.27 ns	0.02*	0.0004*
Subplot error	4	1334.2	0.001	0.76	0.006	0.0002
CV		1.72	0.77	4.22	2.08	3.54

*, ** represents significant at probability levels of 0.05 and 0.01 respectively; ns means non-significant.

Shoot length and diameter, number of leaves per shoot and leaf area

It is evident in Figure 2 that the maximum shoot length observed in NAA (10 and 20 mg/L) and ethephon 50 mg/L at about 30, 29.2 and 29 cm, respectively. A minimum shoot length was observed in control it showed no significant difference with NAA acid 40 mg/L and ethephon 50 mg/L. NAA (10 mg/L) caused the maximum leaf areas at about 10.1 and 10.5 cm in the “on” and “off” year, respectively a minimum leaf area was observed in control at about 5.1 cm in on year (Fig. 3). Maximum number of leaves per shoot observed in NAA (10 and 20 mg/L) and ethephon 50 mg/L at about 29.5, 28 and 28.1 cm, respectively that they showed no significant difference with GA_3 (150 mg/L) in “on” year. Minimum of number of leaves per shoot was observed in control in “off” year (Fig. 3).

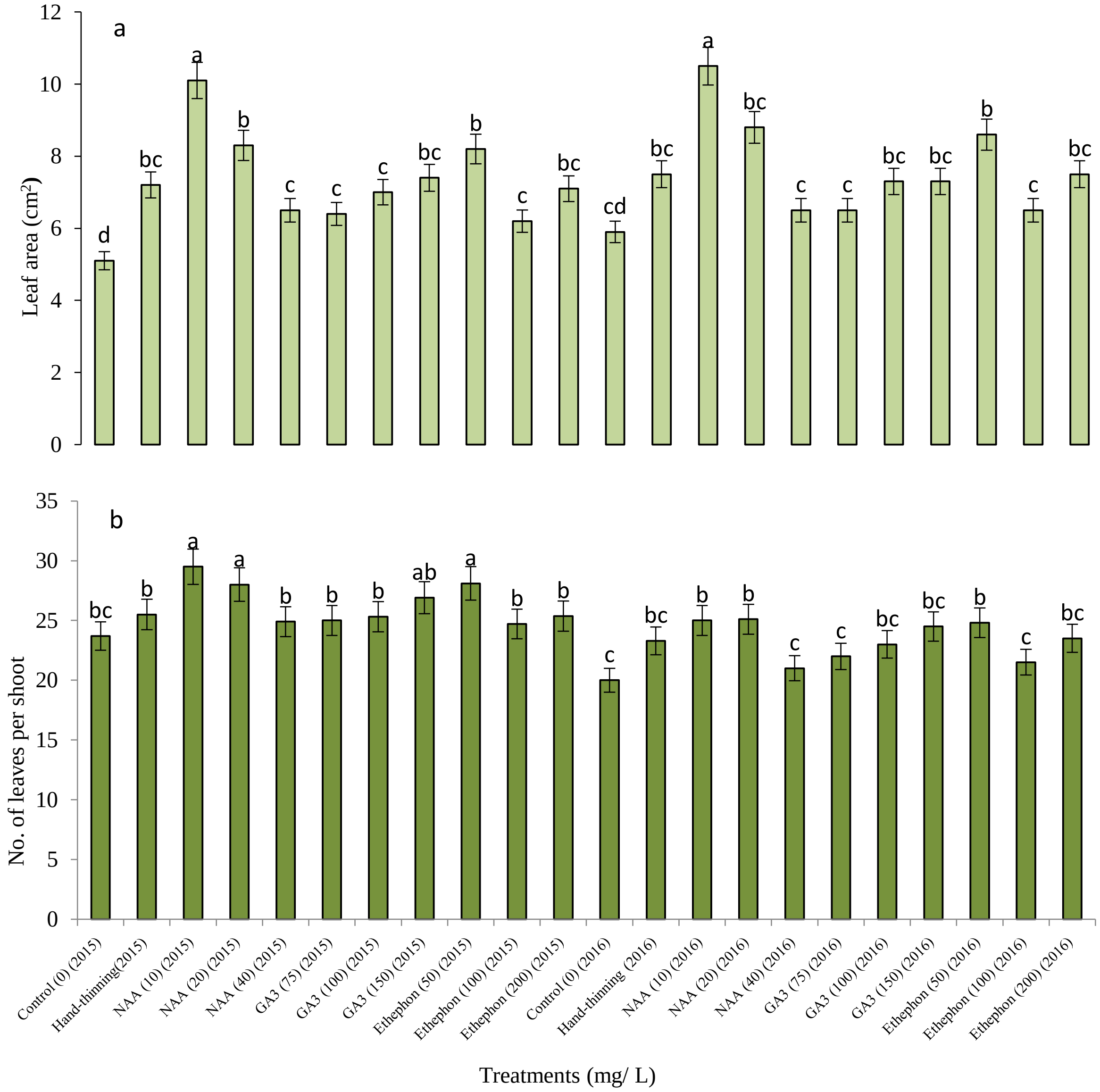

Fig. 3. Effect of different thinning techniques and time on leaf area (a), and No. of leaves per shoot (b) in seedless barberry. Columns with common letters are not significantly different according to Duncan's test.

The starch and sugar of shoots

Figure 4 shows that shoot starch and total sugar content (at the end of the growing season) in the "off" year was significantly more than the "on" year in control and other treatments. Most of the treatments increased starch and total sugar in shoots at the end of the growing season in the "on" year. The lowest percentage of sugar and starch in the shoot was related to control treatment and the maximum percentage of shoot starch was observed in plants treated with NAA (10 and 20 mg/L), ethephon (200 mg/L) and GA3 (150 mg/L), respectively. The highest shoot sugar related to NAA (20 mg/L), ethephon (200 mg/L) and GA_3 (150 mg/L), respectively. Chemical thinning enhanced starch and sugar to a greater extent than hand-thinning.

Chlorophyll content

Figure 5 shows that in the "off" year, leaves chlorophyll content was significantly lower than "on" year in control. It was found that chlorophyll content was decreased with the application of thinning in the "on" year while it was increased in "off" year. In the "on" year, maximum chlorophyll was related to the control and NAA (10 mg/l). Other thinning treatments except hand thinning, GA_3 (75 mg/l) and ethephon (100 mg/l) resulted in a significant reduction of chlorophyll content in seedless barberry leaves. Minimum chlorophyll was observed in plants treated with GA_3 (150 mg/L) and NAA (20 mg/L) treatments, respectively (Fig. 5). In the "off" year, minimum chlorophyll was related to the control and maximum of it was observed in plants treated with NAA (10 mg/L) (Fig. 5).

Morphological traits of berries and shrub yield

Length, width, volume, fresh and dry weight of berries

Results presented in Figure 7 and Table 4 shows that all of the measured morphological traits except fruit dry weight of berries were significantly higher in the "off" year than "on" year. In the "on" year, all thinning treatments significantly increased the length, width and volume of fruits. The highest fruit width related to NAA (10 mg/L) and other treatments showed no significant difference with the control (Fig. 7). Maximum and minimum fruit length was related to the control in "off" and "on" year, respectively. NAA (10 mg/L), hand thinning and ethephon (50mg/L) caused the highest length of fruit in on year. The highest fruit volume was related to control in off year and NAA (10 mg/L) and ethephon (200 mg/L) in on year and the lowest of it related to control in "on" year. Maximum fresh weight of fruits was observed in ethephon (200 mg/L) in "off" year while the maximum dry weight of fruits related to NAA (40 mg/L) that it showed no significant difference with NAA (10 mg/L), and ethephon (200 mg/L) in "on" and a minimum of them were observed in the control in "on" year (Table 4).

Table 4. Effect of different thinning techniques and time on some physical and biochemical traits of seedless barberry fruit.

Year	Treatments	Length (mm)	Volume of 10 berry (cm^3)	Fresh weight of 100 berry (gr)	Dry weight of 100 berry (gr)
2015	Control (0)	7.57 ± 0.06^{d}	3.48 ± 0.06^{c}	14.04 ± 0.07^{c}	3.86 ± 0.03^{d}
	Hand-thinning	8.2 ± 0.06^{bc}	4.44 ± 0.01^{b}	15.88 ± 0.03^{b}	$4.9 \pm 0.06b^{bc}$
	NAA (10)	8.3 ± 0.02^{b}	5.15 ± 0.03^{a}	17.05 ± 0.02^{ab}	5.21 ± 0.06^{ab}
	NAA (20)	8.03 ± 0.02^{c}	4.54 ± 0.01^{b}	15.22 ± 0.06^{bc}	4.66 ± 0.06^{cd}
	NAA (40)	8.04 ± 0.03^{c}	4.74 ± 0.01^{ab}	17.08 ± 0.003^{ab}	5.5 ± 0.1^{a}
	GA_3 (75)	8 ± 0.03^{c}	4.57 ± 0.1^{b}	15.81 ± 0.3^{b}	4.3 ± 0.06^{cd}
	GA_3 (100)	8.1 ± 0.003^{c}	4.9 ± 0.2^{ab}	16.46 ± 0.15^{ab}	4.15 ± 0.009^{cd}
	GA_3 (150)	8.11 ± 0.003^{c}	4.91 ± 0.1^{ab}	16.02 ± 0.03^{b}	4.03 ± 0.2^{d}
	Ethphon (50)	8.17 ± 0.01^{bc}	4.9 ± 0.03^{ab}	16.03 ± 0.04^{b}	4.78 ± 0.01^{bc}
	Ethphon (100)	8.13 ± 0.01^{c}	4.9 ± 0.06^{ab}	17.21 ± 0.06^{ab}	4.94 ± 0.01^{bc}
	Ethphon (200)	8.14 ± 0.3^{c}	5.12 ± 0.1^{a}	17.21 ± 0.07^{ab}	5.21 ± 0.06^{ab}
2016	Control (0)	8.5 ± 0.3^{a}	5.18 ± 0.3^{a}	17.19 ± 0.06^{ab}	4.33 ± 0.1^{cd}
	Hand-thinning	8.3 ± 0.03^{b}	4.97 ± 0.008^{ab}	16.88 ± 0.03^{ab}	4.25 ± 0.2^{cd}
	NAA (10)	8.28 ± 0.02^{b}	5 ± 0.3^{ab}	16.96 ± 0.1^{ab}	4.25 ± 0.1^{cd}
	NAA (20)	8.06 ± 0.06^{c}	4.56 ± 0.03^{b}	15.74 ± 0.06^{b}	3.98 ± 0.06^{d}
	NAA (40)	8.02 ± 0.05^{c}	4.8 ± 0.03^{ab}	17.04 ± 0.05^{ab}	4.29 ± 0.1^{cd}
	GA_3 (75)	8.07 ± 0.01^{c}	4.81 ± 0.09^{ab}	16.08 ± 0.08^{b}	4.05 ± 0.03^{d}
	GA_3 (100)	8.11 ± 0.003^{c}	4.82 ± 0.3^{ab}	16.76 ± 0.22^{ab}	4.27 ± 0.07^{cd}
	GA_3 (150)	8.14 ± 0.003^{c}	4.97 ± 0.1^{ab}	16.5 ± 0.15^{ab}	4.22 ± 0.02^{cd}
	Ethphon (50)	8.15 ± 0.06^{c}	4.96 ± 0.06^{ab}	16.47 ± 0.13^{ab}	4.15 ± 0.06^{cd}
	Ethphon (100)	8.12 ± 0.03^{c}	4.78 ± 0.06^{ab}	17.36 ± 0.06^{ab}	4.37 ± 0.01^{cd}
	Ethphon (200)	8.22 ± 0.03^{bc}	5.06 ± 0.3^{ab}	17.56 ± 0.07^{a}	4.35 ± 0.06^{cd}

Different letter(s) in each row indicates significant differences according to Duncan's multiple range tests at $P < 0.01$.

Table 4. (Continued).

Year	Treatments	Ascorbic acid (mg/100g)	pH	TA (%)	Anthocyanin (mg/l)
2015	Control (0)	1537±3.1^{e}	4.25±0.07^{b}	4.16±0.1^{b}	0.340 ±0.0008^{cd}
	Hand-thinning	1908±0.6^{c}	4.3±0.07^{b}	3.35±0.05^{bc}	0. 399±0.003^{c}
	NAA (10)	2438±3.1^{ab}	4.23±0.07^{b}	3. 75±0.04^{b}	0.4±0.01^{c}
	NAA (20)	2226±0.3^{b}	4.24±0.01^{b}	3.66±0.06^{b}	0.481±0.001^{ab}
	NAA (40)	2226±0.6^{b}	4.22±0.06^{b}	3.38±0.003^{bc}	0.417±0.003^{bc}
	GA_3 (75)	1902±0.6^{c}	4.21±0.03^{b}	3.04±0.01^{c}	0.434±0.001^{bc}
	GA_3 (100)	1918±0.4^{c}	4.22±0.07^{b}	3.33±0.04^{bc}	0.409±0.003^{c}
	GA_3 (150)	2020±0.6^{bc}	4.23±0.07^{b}	3.31±0.03^{bc}	0.410±0.003^{c}
	Ethphon (50)	2120±6b^{b}	4.27±0.006^{b}	3.82±0.02^{b}	0.435±0.001^{bc}
	Ethphon (100)	2385±1.5^{ab}	4.26±0.01^{b}	3.6±0.04^{bc}	0.443±0.0004^{b}
	Ethphon (200)	2332±0.5^{ab}	4.35±0.01^{ab}	3.6±0.05^{bc}	0.503±0.001^{ab}
2016	Control (0)	1688±3.5^{de}	4.32±0.04^{b}	4.99±0.1^{a}	0.405±0.0008^{bc}
	Hand-thinning	2093±2.4^{bc}	4.3±0.08^{b}	4. 1±0.05^{b}	0. 415±0.02^{bc}
	NAA (10)	2521±6^{a}	4.24±0.07^{b}	4.69±0.04^{ab}	0.406±0.01^{bc}
	NAA (20)	2346±5^{ab}	4.22±0.02^{b}	4.5±0.06^{ab}	0.502±0.01^{ab}
	NAA (40)	2319±4^{ab}	4.22±0.06^{b}	4.17±0.003^{b}	0.478±0.003^{ab}
	GA_3 (75)	2024±6^{bc}	4.21±0.03^{b}	4.04±0.01^{b}	0.498±0.009^{ab}
	GA_3 (100)	2099±6^{bc}	4.22±0.07^{b}	4.21±0.04^{b}	0.456±0.01^{b}
	GA_3 (150)	2183±8^{b}	4.23±0.07^{b}	4.3±0.03^{ab}	0.41±0.003^{bc}
	Ethphon (50)	2184±1.5^{b}	4.26±0.01^{b}	4.68±0.02^{ab}	0.501±0.0004^{ab}
	Ethphon (100)	2391±1.5^{ab}	4.28±0.003^{b}	4.5±0.04^{ab}	0.510±0.0004^{a}
	Ethphon (200)	2386±2.5^{ab}	4.4±0.03^{a}	4.5±0.05^{ab}	0.520±0.01^{a}

Shrub yield

Hand and chemical thinning were done in “on” year (2015) and caused to decrease in yield in hand thinning treatment but, didn’t decrease yield in other treatments and control in the same year. This indicated that hand thing isn’t suitable in on year because of decreased yield while chemical thinning is suitable because it caused no difference in yield in comparison to the control. On the other hand, all thinning treatments induced a significant increase in yield of the “off” year. The lowest shrub yield (13980 g) was observed in the hand-thinning treatment in “on” year while the minimum yield was observed in the control (1793g) in “off” year. It is noticeable that the entire treatments improved seedless barberry shrub yield in the “off” year compared to the control this means approaching a balanced bearing every year (Fig. 6).

Biochemical traits of berries

Ascorbic acid (Vitamin C), and pH

Table 4 shows that all thinning treatments significantly improved ascorbic acid content versus control in both years. The highest amount of ascorbic acid was observed in NAA (10 mg/L) in “off” and “on” year, respectively they showed no significant difference with ethephon 100 and 200 mg/L in both years and NAA (20 and 40 mg/L) in “off” year. The lowest amount of ascorbic acid was observed in the control in the “on” and “off” year. Table 4 indicates that the amount of fruit pH in all treatments except ethephon (200mg/l) was not significantly different in comparison to the control.

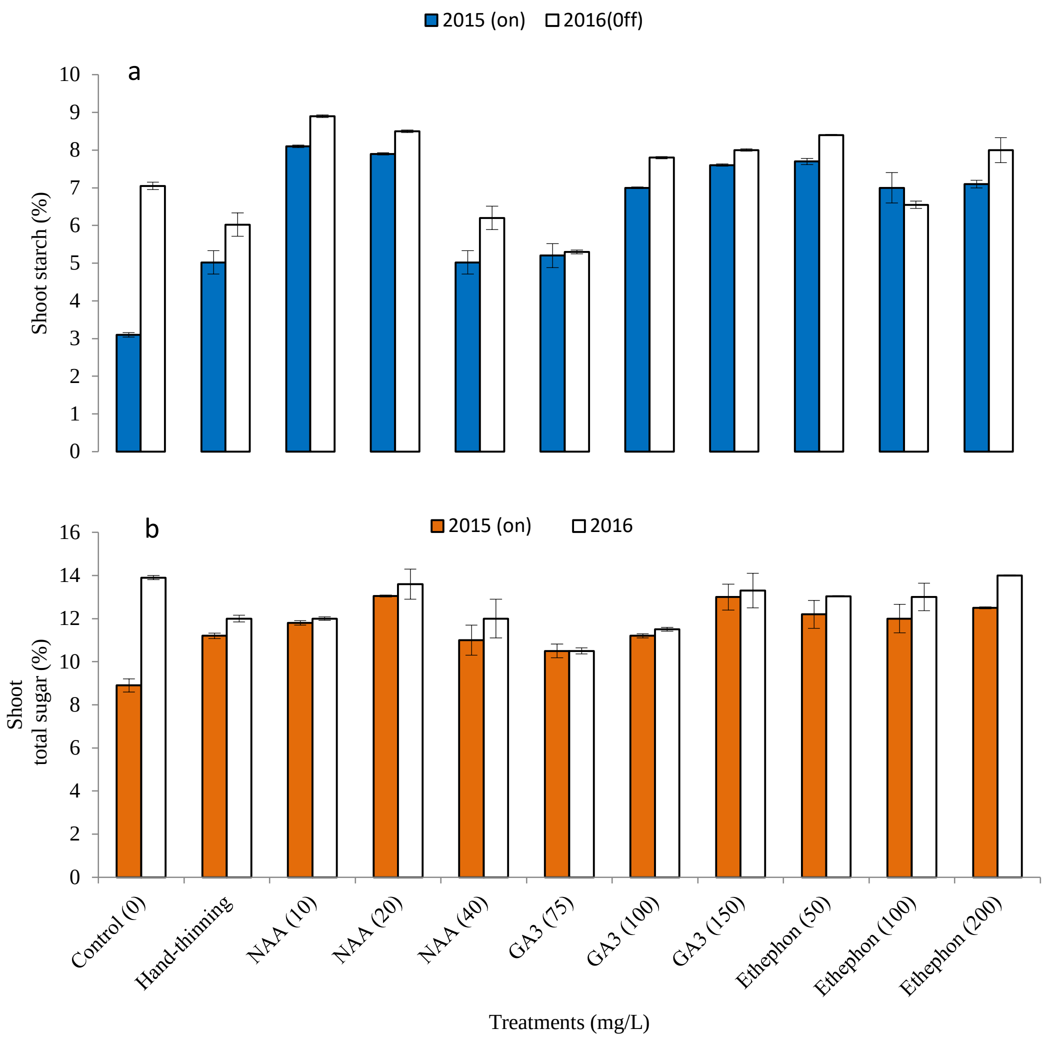

Fig. 4. Effect of different thinning techniques and time on shoot starch (a), and total sugar content (b) in seedless barberry.

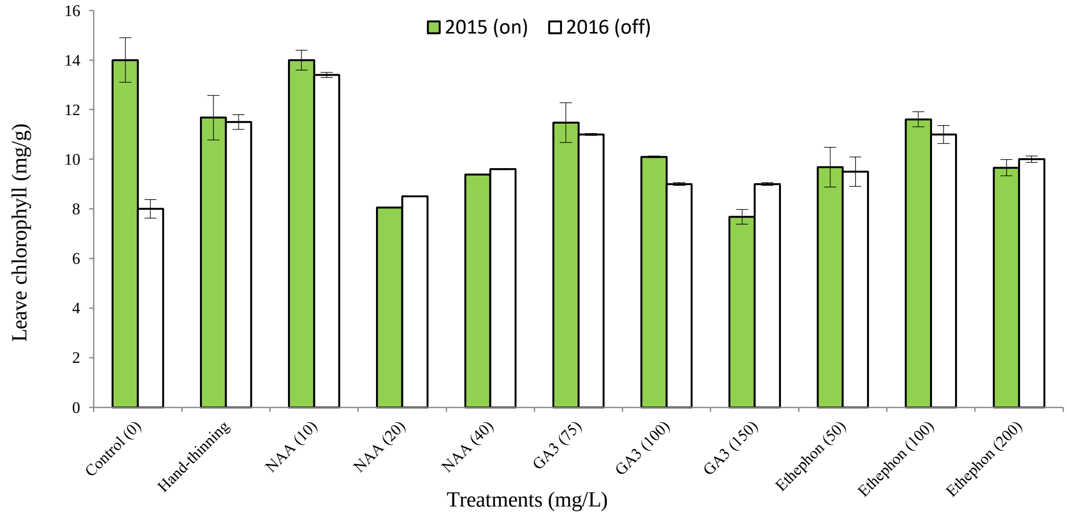

Fig. 5. Effect of different thinning techniques and time on leave chlorophyll content in seedless barberry.

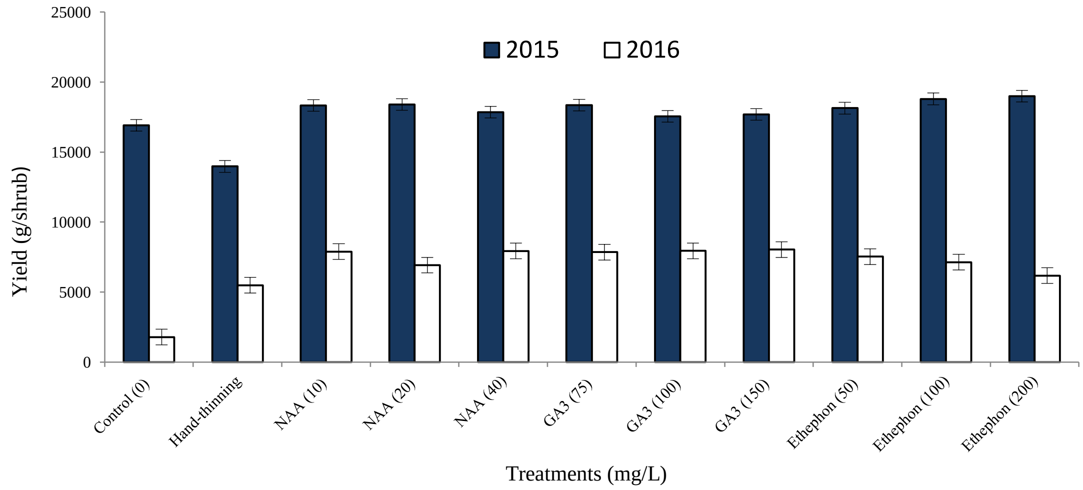

Fig. 6. Effect of different thinning techniques and time on shrub yield in seedless barberry. Columns with common letters are not significantly different according to Duncan's test.

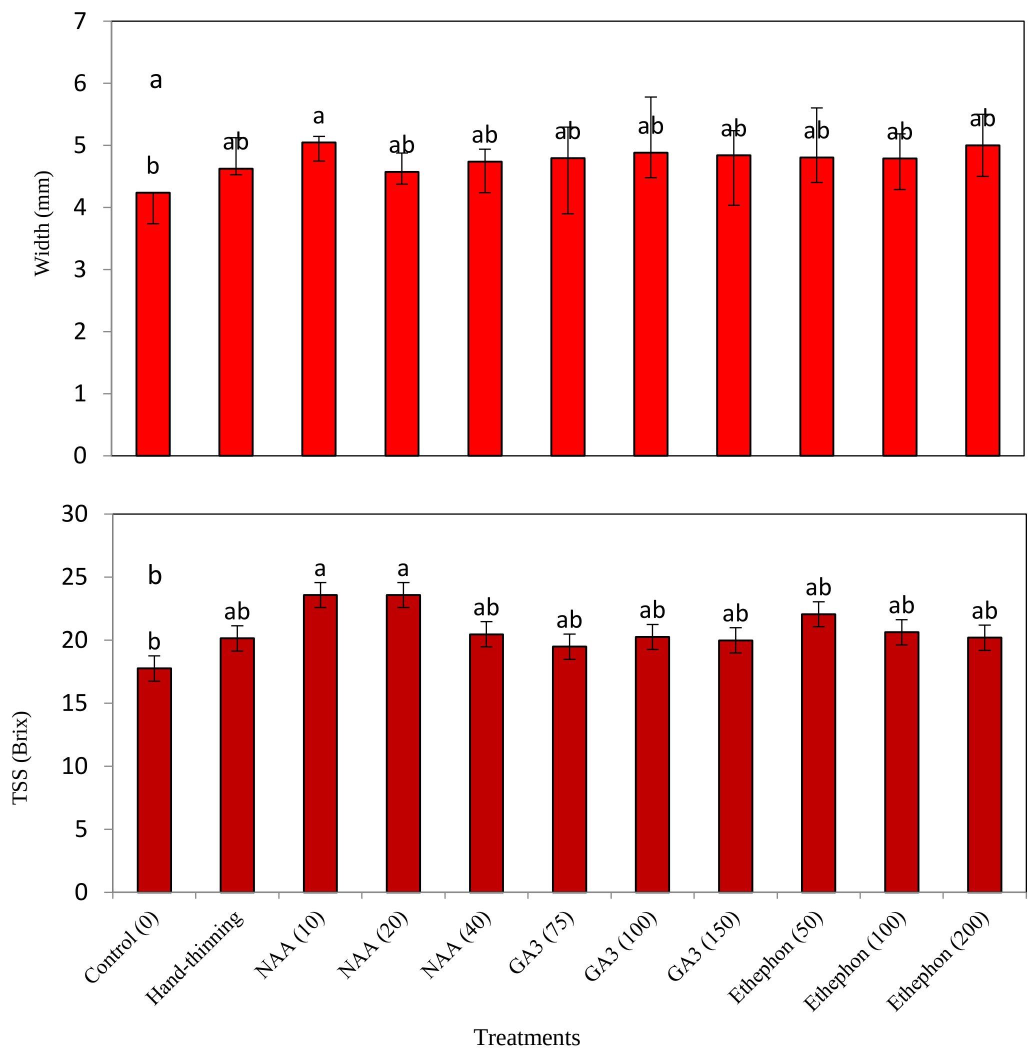

Fig. 7. Effect of different thinning techniques on fruit width (a), and total soluble content (TSS) (b) of seedless barberry. Columns with common letters are not significantly different according to Duncan's test.

Total soluble solids (TSS) and titratable acidity (TA)

According to Figure 7 and Table 4, the treatments resulted in higher total soluble solids and lower total acidity of barberries than the control, respectively. The highest total soluble solid was observed in NAA (10 and 20 mg/L) at about 23.57% Brix that they showed no significant difference with other treatments and the lowest one was related to control at about 17.75% Brix (Fig. 7). Table 4 indicates maximum titratable acidity was related to control at about 4.99 % in the "off" year and the minimum was related to GA_3 (75 mg/L) at about 3.04 % in "on" year.

Fruit anthocyanin

The results in Table 4 showed that the anthocyanin content of berries was considerably higher in the "off" year than "on" year. The lowest amount of anthocyanin content was observed in control and the highest one was observed in ethephon (100 and 200 mg/L) in "off" year it showed no significant difference with ethephon (200 mg/L) and NAA (20 mg/L) in on year and NAA (20 and40 mg/L), GA_3 (75 mg/L) and ethephon (50 mg/L) in the "off" year (Table 4).

DISCUSSION

Application of chemical thinning in this experiment showed that NAA and ethephon were more effective on fruit drop in lower concentrations, while the highest fruit drop was related to the highest rate of GA_3. Taghipure et al. (2011) showed that the application of NAA resulted in fruit thinning of apricot. NAA application disrupts the hormone balance in plants and stimulates ethylene synthesis, so this chemical thinning substance indirectly increases fruit drop (Bertelsen, 2002). It has been shown that seedless barberry yield fluctuates around 40% during five years of study which indicates the biennial bearing phenomena (Rezaei & Balandary, 2011). Seedless barberry morphological traits increased in all thinning treatments and especially in lower concentrations of NAA and ethephon, and higher concentrations of GA_3. The loss of fruit could be one of the important reasons for higher leaf area in barberry.

This experiment showed that chlorophyll content decreased with the application of thinning in the "on" year while it increased in "off" year. In the "on" year chlorophyll content was decreased significantly in most thinning treatments except in hand thinning, GA_3 (75 mg/L), ethephon (100 mg/L) and NAA (10 mg/L). Chlorophyll plays an important role in sunlight absorption and photosynthesis. It has been indicated that chlorophyll content is lower in low-yielding shrubs than in high-yielding shrubs (Choi et al., 1997).

Most of the treatments especially NAA treatment increased starch and total sugar in shoots at the end of the growing season in the "on" year. The loss of fruit after thinning decreased different sinks and consequently, assimilates such as starch and carbohydrate were stored in shoots or remaining fruitlets. On the other hand, leaf area was increased after thinning and assimilated more carbohydrates and stored them in shoots. In shrubs with high carbohydrate storage, more fruits will be produced in the subsequent year and alternate bearing will be decreased while the loss of carbohydrate reserve because of higher yield increases alternate bearing (Spann et al., 2008).

The results of this experiment showed that the orchard management of fruitlet thinning by chemical thinner reagents can increase the yield of seedless barberry, particularly in "off" years. In the "on" year, all the treatments except hand thinning increased the yield. However, hand thinning enhanced the yield even in the "off" year as well. It has been reported that GA_3 could improve fruit set, plant growth, flowering and as a result, increase yield (Williamson &

NeSmith, 2007; Zang et al., 2016). Milić et al. (2018) reported that NAA increased the number of berries per cluster and yield per shrub in blueberries.

Evaluation of different fruit traits showed that chemical thinning was more effective than hand thinning compared to the control and also NAA and ethephon had more positive effects on the improvement of these fruit traits than GA_3. GA_3 at the concentration of 50 mg/L showed the most effective as a thinner chemical on *Prunus salicina* and improved fruit size, colour, TSS and fruit firmness (Erogul & Fatih Sen, 2015). According to Suzuki et al. (1998), fruit weight in blueberries is positively correlated with shoot vigour and the number of leaves that increased morphological fruit traits by chemical thinning. On the other hand, earlier and greater expansion of the LA, which might be the case with NAA treatment, could be the reason for the increased fruit size in barberry. In our study application of NAA and ethephon confirm it. Due to the fruit's abscission after NAA and ethephon application, fewer fruits remained on trees until harvest time, the more photosynthetic resources lead to the larger fruits with higher dry weight. Blueberry hand-thinning and NAA caused a significant increase in fruit fresh and dry weight (Gough et al., 1976). Foliar application of ethephon (100 mg/L) on tangerine (Gallash, 1978) increased fruit size by around 65% which is consistent with our findings.

Evaluation of different biochemical fruit traits showed that ascorbic acid content and total soluble solids were increased under thinning treatments. The role of chemical thinning treatments on increasing the total soluble solids of fruit juice is associated with an increase in the ratio of leaf to fruit after fruit thinning. In this experiment, leaf area was increased after fruitlet abscission and as a result, more assimilates reached the remaining fruits which resulted in higher soluble solids and sweeter berries. Moreover, auxins stimulated the expansion and growth of cells, resulting in leaf area expansion. The increase in total soluble solids and ascorbic acid after hand thinning has been reported in grapes by Singh (1995). Measurement of total acidity showed that thinning treatments decreased total acidity in fruit juice. The application of 20 mg/L NAA on blueberries increased the ratio of total soluble solids to total acidity in its juice (Gough et al., 1976).

Fruit anthocyanin was increased in all treatments which ethephon (200 mg/L) being the most effective one. Anthocyanin is considered one of the indicators for quality assessment in seedless barberry (Rezvani Moghaddam et al., 2013). Ban et al. (2007) revealed that ethephon could promote the fruit ripening process in blueberry. In another study on grapes, it was reported that the colour synthesis decreased when the number of branches was increased (Choi et al., 1997). The increase in anthocyanin pigment after hand or chemical thinning could be related to the increase of soluble solids in fruit. Also, Whiting et al. (2006) found that hand thinning of sweet cherry increased the fruit's red colour and Guidoni et al. (2002) found that hand thinning of bunch of grape increased anthocyanin and flavonoid in berries it is speculated that the interference with other endogenous hormones, the transient rise of ABA, the inhabitation or down-regulation of IAA export from fruitlets after the application of thinning chemicals such as NAA, ethephon or BA initiate the correlatively triggered response and eventually fruitlet abscission (Bangerth, 2000).

CONCLUSION

Alternative bearing is a frequent problem in seedless barberry production. To avoid it, thinning has been employed as a common cultural practice in orchard management. In this study vegetative traits such as shoot length, the number of leaves per shoot and leaf area showed significant increases under the treatments while shoot diameter show no significant difference with control. Chemical thinning remarkably enhanced the starch and sugar of the shoots, especially in on year. The minimum chlorophyll content in “off” year was observed in control and the highest by NAA at 10 ppm. All the treatments increased seedless barberry shrub yield in the “off” year (2016). Biochemical traits such as ascorbic acid, total soluble sugar, total acidity and anthocyanin improved in most treatments. So, the application of NAA 10 mg/l is recommended for control of alternative bearing and fruit quality. In conclusion, we have revealed that the exogenous application of chemical thinning significantly reduced the alternate bearing in barberry. In both years, 2015 and 2016, the quality of berries was improved markedly by treatments compared to the control. NAA at 10 mg. L^{-1} showed the most promising results in barberry. Chemical thinning especially by NAA which is inexpensive and straightforward to use, offers a suitable alternative to reduce the cost of seedless barberry production and will boost farmers’ economic benefits.

Conflict of interest

Authors declare that they have no conflict of interest.

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REFERENCES

Ahmadi, K., Abedzadeh, H.R, & Hatami, F. (2021). Agriculture Statistics. V3. *Horticulture Crops. Ministry of Agriculture Publication*, 164p.

Alemardan, A., Asadi, W., Rezaei, M., Tabrizia, L & Mohammadi, S. (2013). Cultivation of Iranian seedless barberry (*Berberis integerrima* ‘Bidaneh’): A medicinal shrub. *Industrial Crops & Product*, *50*, 276-287. https://doi.org/10.1016/j.indcrop.2013.07.061

Alimirzaee, P., Gohari, A.R., Hajiaghaee, R., Mirzaee, S., Jamalifar, H., Monsef-Esfahani, H.R., Amin, G., Saeidnia, S & Shahverdi, A.R. (2009). 1-Methyl malate from Berberisintegerrima fruits enhances the antibacterial activity of ampicillin against Staphylococcus aureus. *Phytotherapy Research*, *23*, 797-800. https://doi.org/10.1002/ptr.2641

Balandary, A. (1995). The influences of GA3 and ethephon on characteristics of fruit and simplicity of harvesting in seedless barberry. *MSc Thesis. University of Shiraz*.

Ban, T., Kugishima, M., Ogata, T., Shiozaki, S., Horiuchi, S & Ueda, H. (2007). Effect of ethephon (2-chloroethylphosphonic acid) on the fruit ripening characters of rabbiteye blueberry. *Scientia Horticulture*, *112*(3), 278-81. https://doi.org/10.1016/j.scienta.2006.12.027

Bangerth, F. (2000). Abscission and thinning of young fruit and their regulation by plant hormones and bioregulators. *Plant Growth Regulators*, *31*, 43-59. https://doi.org/10.1023/A:1006398513703

Bertelsen, M.G. (2002). Banzyl adenine and other thinning agents for pear cv. 'Clara Frijs'. *Journal of the American Pomological Society*, 56 (3): 149-155. https://doi.org/10.21273/JASHS.117.5.775

Choi, I.M., Lee, H.C, Yun, C.J, Lee, C.H. (1997) Influence of number of clusters per vine on vine growth and fruit quality in 2-year-old 'Kyoho' grape (*Vitis labruscana* B.). *Journal of Horticulture Science*, *39*(1), 134-139.

Costa, G., Botton, A., & Vizzotto, G. (2019). Fruit Thinning: Advances and Trends. In: Warrington I. (ed) Horticultural Reviews, 46. *John Wiley & Sons, New Jersey*, pp 185-226.

Easlon, H.M & Bloom, A.J. (2014). Easy Leaf Area: Automated digital image analysis for rapid and accurate measurement of leaf area. *Applications in Plant Sciences*, *2*(7), 1-4. https://doi.org/10.3732/apps.1400033.

Ebrahimi-Mamaghani, M., Arefhosseini, S.R., Golzarand, M., Aliasgarzadeh, A & Vahed-Jabbary, M. (2009). Long-term effects of processed *Berberis vulgaris* on some metabolic syndrome components. Iranian *Journal of Endocrinology and Metabolism*, *11*(1), 41-47.

Erogul, D & Fatih Sen, F. (2015). Effects of gibberellic acid treatments on fruit thinning and fruit quality in Japanese plum (*Prunus salicina* L.). *Scientia Horticulture, 186*, 137-142. https://doi.org/10.1016/j.scienta.2015.02.019

Gallash, P.T. (1978). Thinning imperials mandarin with ethephon increases fruit size and grower returns. *Proceeding of International Society for Citriculture*, 1, 276-279.

Gough, R.E, Shutak, V.G & Windus, N.D. (1976). Observations on the vegetative and reproductive growth of the cultivated high shrub blueberry. *HortScience*, *11*, 260-261. https://doi.org/10.21273/HORTSCI.11.3.260

Guidoni, S., Allara, P., & Schubert, A. (2002). Effect of cluster thinning on berry skin anthocyanin composition of *Vitis vinifera* cv. Nebbiolo. *American Journal of Enology and Viticulture*, *53*(3), 224-226.

Hashemzaei, M., Qaredashi, R., & Hosseinzadeh, H. (2012). Inhibitory effect of Berberis vulgaris aqueous extract on acquisition and reinstatement effects of morphine in conditioned place preferences (CPP) in mice. *Research in Pharmaceutical Sciences*, *7*(5), 839. https://doi.org/10.17795/jjnpp-16145.

Hedge, J.E, Hofreiter, B.T. (1962). Estimation of starch by anthrone reagent. In: Whistler RL and Be-Miller JN (Ed.) Methods in carbohydrate chemistry, Academic Press, New York, 220-225.

Kafi, M., Balandary, A., Rashed-Mohasel, M.H., Koochaki, A & Molafilabi, A. (2002). Berberis: Production and Processing. Zaban & Adab Press, Iran, 209 p.

Kassem, H.A., Al-Obeed, R.S., Ahmed, M.A., & Omar, A.K. (2011). Productivity, fruit quality and profitability of jujube trees improvement by preharvest application of agro-chemicals. *Middle-East Journal of Science Research*, *9*(5), 628-637.

Kochert, G. (1978). Carbohydrate determination by the phenol-sulphuric acid method. In: Hellebust JA, Craigie JS (Ed.) Handbook of Phycological Methods, Physiological and Biochemical Methods. *Cambridge University*, *London*, pp 95–97.

Lichtenthder, H.K. (1987). Chlorophylls and carotenoids: Pigments of photosynthetic bio membranes. *Methods in Enzymology*, *148*, 350-382. https://doi.org/10.1016/0076-6879(87)48036-1

Milić, B., Tarlanović, J., Keserović, Z., Magazin, N., Miodragović, M., & Popara, G. (2018). Bioregulators can improve fruit size, yield and plant growth of northern highbush blueberry (*Vaccinium corymbosum* L.). *Scientia Horticulturae*, 235, 214-220. https://doi.org/10.1016/j.scienta.2018.03.004

Ranjana, S. (1986). Hand book of analysis and quality control for fruit and vegetable products. Second ed. *Tata Mc GroW-Hill, Newdelhi*. 1112 pages.

Rezaei, M. & Balandary A. (2011) Alternate bearing in barberry, hypothesis and solution. In: Proceeding of the *First National Conference on Seedless Barberry and Jujube, Birjand, Iran*.

Rezvani Moghaddam, P., Fallahi, J., Aghhavani Shajari, M., & Nassiri Mahallati, M. (2013). Effects of harvest date, harvest time, and postharvest management on quantitative and qualitative traits in seedless barberry (*Berberis vulgaris* L.). *Industrial Crops and Products*, *42*, 30-36. https://doi.org/10.1016/j.indcrop.2012.05.007

Sengul, M., Ercisli, S., Yildiz, H., Gungor, N., Kavaz, A., & Çetin, B. (2011). Antioxidant, antimicrobial activity and total phenolic content within the aerial parts of *Artemisia absinthum*, *Artemisia santonicum* and *Saponaria officinalis*. *Iranian Journal of Pharmaceutical Research*, *10*(1), 49.

Shidfar, F., Ebrahimi, S. S., Hosseini, S., Heydari, I., Shidfar, S., & Hajhassani, G. (2012). The effects of Berberis vulgaris fruit extract on serum lipoproteins, apoB, apoA-I, homocysteine, glycemic control and total antioxidant capacity in type 2 diabetic patients. *Iranian Journal of Pharmaceutical Research*, *11*(2), 643.

Singh, S. (1996). Ripening and quality of grape (*Vitis vinifera* L.) as affected by cluster thinning. *In Horticultural Abstracts*, 66, 1, 9-15.

Spann, T. M., Beede, R. H., & DeJong, T. M. (2008). Seasonal carbohydrate storage and mobilization in bearing and non-bearing pistachio (*Pistacia vera*) trees. *Tree Physiology*, *28*(2), 207-213. https://doi.org/10.1093/treephys/28.2.207.

Suzuki, A., Shimizu, T., & Aoba, K. (1998). Effects of leaf/fruit ratio and pollen density on highbush blueberry fruit quality and maturation. *Journal of the Japanese Society for Horticultural Science*, *67*(5), 739-743. https://doi.org/10.2503/jjshs.67.739

Taghipour, L., Rahemi, M., & Assar, P. (2011). Thinning with NAA, NAD, ethephon, urea and by hand to improve fruit quality of 'Gerdi' apricot. *Brazilian Journal of Plant Physiology*, *23*, 279-284. https://doi.org/10.1590/S1677-04202011000400005 .

Taghipure, M & Rahemi, M. (2009). Effect of several chemical thinning on thinning percentage and quality of Apricot fruits (*Prunus armenica* L. cv. 'Khiary'). *Journal of Horticultural Science*, *23*(2), 78-84.

Vijayan, K., Chakraborti, S. P., Ercisli, S., & Ghosh, P. D. (2008). NaCl induced morpho-biochemical and anatomical changes in mulberry (*Morus spp*.). *Plant Growth Regulation*, *56*(1), 61-69. https://doi.org/10.1007/s10725-008-9284-5

Whiting, M. D., Ophardt, D., & McFerson, J. R. (2006). Chemical blossom thinners vary in their effect on sweet cherry fruit set, yield, fruit quality, and crop value. *HortTechnology*, *16*(1), 66-70. https://doi.org/10.21273/HORTTECH.16.1.0066.

Williamson, J. G., & NeSmith, D. S. (2007). Effects of CPPU applications on southern highbush blueberries. *HortScience*, *42*(7), 1612-1615. https://doi.org/10.21273/HORTSCI.43.5.1446

Wrolstad, R. E. (1976). Color and pigment analyses in fruit products. Station Bulletin 624, Agricultural Experimental Station. *Oregon State University*, *Corvallis*, *OR*, *USA*.

Zang, Y. X., Chun, I. J., Zhang, L. L., Hong, S. B., Zheng, W. W., & Xu, K. (2016). Effect of gibberellic acid application on plant growth attributes, return bloom, and fruit quality of rabbit eye blueberry. *Scientia Horticulturae*, *200*, 13-18. https://doi.org/10.1016/j.scienta.2015.12.057

Zargari, A. (1990). Medicinal Plants. Fourth ed. *Tehran University, Iran*.